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Evolution of Gravity Receptors in the Ear**

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Background

Sensory hair cells of the vestibular system of the inner ear have been divided into type I and the type II cells (see original proposal for references). These cells differ in a number of ways. The location of type I and type II cells differ from one another in each of the endorgans of the ear, and it has been suggested that the two types have functional differences. Although type II hair cells are considered to be ubiquitous among all vertebrates, it has been argued that type I hair cells are only found in amniotes (mammals, birds and reptiles) and not in anamniotes (jawless fish, cartilaginous fish, bony fish, amphibians). Our recent work, however, shows that sensory hair cells very much like the type I hair cell are present in the vestibular endorgans of the ears of a fish. These findings suggest to us that there is a complex organization to the otolithic receptors of the fish ear, and that this organization that may have significant similarities to the organization of mammalian otolithic endorgans. It is important to note that there is great structural and functional similarity between the vestibular endorgans of the ears of fishes (sacculae, utricle, lagena, cristae) and those of mammals.

Work Done with NASA Support

Studies over the past several years have demonstrated the presence of two types of hair cells in the ears of a teleost fish, *Astronotus ocellatus*, the oscar (Saidel et al, 1990a, b; Chang et al, 1992; Popper et al, 1993). This has led to questions of the origins and evolution of two hair cell types. Over the past year we have made several significant findings that extend our earlier observations, and suggest an earlier origin of two cell types than we had predicted in the past.

(1) Hair Cell Types in the Sacculae of the Goldfish (Lanford and Popper, 1993, 1994; Saidel et al, 1995): Investigations of the goldfish, *Carassius auratus*, have demonstrated that multiple hair cell types are found in all of the otolithic endorgans. We demonstrated, using transmission electron microscopy and several immunohistological techniques, that the cells in the rostral end of the sacculae are type I-like, while those at the caudal end are type II cells. We have further extended this work to demonstrate that the dichotomy of hair cell types found in the utricle of the oscar is also found in the goldfish. Interestingly, while there are some differences in details of the structure of the type I-like cells in the sacculae vs the utricle of both the oscar and goldfish, the utricular cells in both species very much resemble one another.

The finding of two types of hair cells in the sacculae of the goldfish has a number of important ramifications. First, these findings strongly support our hypothesis that type I-like hair cells are found in broadly distributed taxonomic groups of fishes. The goldfish is taxonomically very distinct from the oscar, and their common ancestor lies far back in the evolution of fishes. Assuming that the type I-like hair cell has only evolved once in the course of evolution, this suggests to us that type I-like cells arose early in the evolution of fishes (or perhaps even earlier).

A second important outcome of this work is its implications for understanding how the vestibular system in fishes responds to acoustic or vestibular stimulation. A number of studies have demonstrated that there is some variation in the response characteristics of the rostral and caudal regions of the goldfish sacculae. Moreover, patch-clamp studies of hair cells from the goldfish sacculae have shown that there are two, or perhaps, three, cell types (as defined by the presence of different types of channels in the cell membrane). Our investigations are the first to provide the first combined ultrastructural and physiological base for these observations. More importantly, our

results strongly suggest that there is functional dichotomy within the goldfish sacculus.

The results from our laboratory, combined with the data on eighth nerve physiology and patch-clamping, strongly suggest that the two types of hair cells (type I-like and type II) are functionally dichotomous. If this turns out to be correct, it means that the vestibular organs of fishes (and perhaps sharks and agnathans) have receptors with different response characteristics that may serve to extract different features from various signals impinging upon the ear. Furthermore, this means that the vestibular system of fishes is functionally far more complex than heretofore thought, and that such systems could serve as ideal models for investigation of vertebrate vestibular function.

(2) Multiple Hair Cell Types in the Lateral Line (Song, Jia and Popper, 1994; Song, Yan and Popper, 1994, 1996): The lateral line is ubiquitous among fishes. It is located on body surface and provides for detection of hydrodynamic signals impinging upon a fish. The lateral line system contains sensory hair cells that are very similar to those found in the ear, and there is reason to believe that the ear and lateral line share some common ancestor structure in the early evolution of vertebrates. Thus, if the lateral line has two hair cell types, as does the ear, the implication is that the presence of multiple hair cell types, and multiplicity of function arose far earlier in the evolution of vertebrates than even indicated by their presence in the ear of fishes.

In order to test this hypothesis we have started to examine the lateral line system of one of our main experimental animals, the oscar, *Astronotus ocellatus*. In the first part of this study, which is now being completed, we have studied the lateral line using gentamicin sulphate, an ototoxic drug that destroys type I-like hair cells but that does not appear to damage type II hair cells.

Fish were treated with very low doses of gentamicin and the hair cells of the lateral line were examined from 1 to 12 days later using scanning electron microscopy (SEM). Our results demonstrated that the hair cells found in neuromasts of lateral line canal organs were totally destroyed within 1 day of treatment, while the hair cells in free neuromasts were undamaged after 12 days of treatment. While we have yet to do studies using S-100 and TEM, the results strongly imply that there are differences in the hair cells of the canal and free neuromast parts of the lateral line. This finding, unto itself, is critically important since we have demonstrated, for the first time, some differences in hair cells of this endorgan. Our hypothesis is that the gentamicin-sensitive cells of the canal neuromasts are type I-like and those of the free neuromasts are type II. If this proves to be the case in our next studies, it will mean that the two hair cell types evolved very early in the origin of vertebrates. Such results would also have profound implications on questions pertaining to lateral line function.

Several additional results have emerged from the lateral line studies. First, and perhaps most important, we have now demonstrated that the hair cells in the canal neuromasts are regenerated several days after cell damage. While other investigators have shown that whole neuromasts of the lateral line can regenerate, this is the first demonstration of regeneration of individual cells.

Second, hair cells of the canal neuromasts regenerate in the presence of the ototoxic drug. This has heretofore never been demonstrated in any vertebrate species. However, we have also found that regeneration only continues up to a certain hair cell 'stage' when the ototoxic drug is present, suggesting to us that the drug only effects hair cells when they develop certain, as yet undefined, characteristics. Initial evidence suggests that the type I-like cells, which are damaged by

the drug, may actually go through a type II cell stage (such cells are not damaged by the drug). This observation has significance for questions of the development and origin of multiple hair cell types.

(3) Nerve Calyx in Fish Ears (Lanford and Popper, 1995, 1996). One of the characteristics that has been thought to be closely related to amniote type I hair cells is the specialized nerve ending, the calyx, which surrounds the bulk of the hair cell body. While the function of the calyx is not known, it has been ascribed a variety of functions. It has also been one of the defining characteristics of type I cells. Moreover, it has never been found in any vertebrates other than birds, mammals and reptiles, and the calyx in reptiles is often only partial.

Our studies have now demonstrated that the calyx is not unique to amniotes and that it is present at least in the cristae of the semicircular canals in goldfish. The calyx in goldfish envelopes a number of cells, has efferent endings on it, and envelopes the lower portion of the hair cells. This is a pattern that is very similar to that found in reptiles.

These results strongly suggest that the calyx is far older in terms of the evolution of the ear than previously thought. While we know nothing of its function in fishes, this may be a good system in which to study these structures.

Significance

The results of our studies have demonstrated that: (1) there are multiple hair cell types in the vestibular endorgans of the ear of fishes, (2) these hair cell types are very similar to those found in the mammalian vestibular endorgans, (3) the nerve calyx is also present in fishes, and (4) multiple hair cell types and the calyx have evolved far earlier in the course of vertebrate evolution than heretofore thought.

Understanding the structure of the vestibular endorgans has important implications for being able to understand how these organs respond to gravistatic, acceleration and acoustic input. While it is becoming clear that different hair cell types in the mammalian vestibular endorgans may play different roles in signal transduction, and therefore send different types of information about vestibular signals to the brain, almost nothing is known about the specific response properties of the different types of hair cells. The difficulty in analysis of the function of different hair cell types in mammalian vestibular endorgans lies, in part, with the fact that the two cell types are primarily intermingled in each endorgan, and it is very hard to isolate one cell type from another for physiological investigations. Moreover, type I cells in mammals are surrounded by a nerve chalice that may complicate the response of the cell, and make it more difficult to analyze the function of the cell itself.

The vestibular endorgans of fishes may provide an ideal system in which to analyze functional differences in hair cells. Not only are the two hair cell types similar to those found in mammals, they are located in very discrete regions in each endorgan. Thus, it is relatively easy to gain access to cells of one or the other type. Moreover, since type I-like hair cells in fish do not have a nerve chalice, it is potentially easier to study the response of this cell without the complications of a nerve ending that is poorly understood.

The presence of the two cell types in the lateral line have equally significant implications for

studies of the vestibular system. While the function of the lateral line is not vestibular, the very easy access to these cells, as compared to access to the cells of the ear, means that they could be used in studies that provide basic insights into the function of cells that are similar to those found in the mammalian vestibular system.

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